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(54) Title: REGULATION OF HUMAN DIPEPTIDYL-PEPTIDASE IV-LIKE ENZYME

(57) Abstract: Reagents which regulate human dipeptidyl-peptidase IV-like enzyme and reagents which bind to human dipeptidyl-peptidase IV-like enzyme gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, tumors and peripheral and central nervous system disorders including pain and neurodegenerative disorders.

REGULATION OF HUMAN DIPEPTIDYL-PEPTIDASE IV-LIKE ENZYME

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of regulation of human dipeptidyl-peptidase IV-like enzyme to provide therapeutic effects.

BACKGROUND OF THE INVENTION

Dipeptidyl peptidase IV is a post-proline cleaving enzyme which will remove the di-peptides Xaa-Pro (Xaa is any amino acid) from the N-terminus of proteins or polypeptides. Dipeptidyl peptidase IV has been found in a variety of mammalian cells and tissues including kidney, placenta, blood plasma, and neurological tissues. Dipeptidyl peptidase IV has been implicated as being involved in or associated with a number of conditions and diseases, including peripheral and central nervous system disorders including pain and neurodegenerative diseases and tumor angiogenesis. For example, the level of dipeptidyl peptidase IV activity has been found to be higher in astrocytomas, gliomas, and immature human central nervous system tissue when compared to dipeptidyl peptidase IV activity in normal, adult human brain, suggesting that dipeptidyl peptidase IV may play a role in the development of the central nervous system as well as the development of neurodegenerative tumors (1, 2, 3). The neurotransmitter neuropeptide Y of sympathetic neurons is converted by di-peptidyl peptidase IV activity into a form that serves as an angiogenic agonist (4). Dipeptidyl peptidase IV may therefore participate in the development of tumors in sympathetic nerves. It has also been suggested that dipeptidyl peptidase IV activity contributes to the conversion of neuropeptides that are involved in neurodegenerative conditions (5). Regulation of dipeptidyl peptidase IV activity may prove critical for treating neuropeptidyl activation the leads to angiogenesis or neurodegeneration. Thus, there is a need in the art for identifying new dipeptidyl peptidase IV enzymes and methods of regulating their activities.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human dipeptidyl-peptidase IV-like enzyme. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a dipeptidyl-peptidase IV-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 55% identical to the amino acid sequence shown in SEQ ID NO: 8; and
the amino acid sequence shown in SEQ ID NO: 8.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a dipeptidyl-peptidase IV-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 55% identical to the amino acid sequence shown in SEQ ID NO: 8; and
the amino acid sequence shown in SEQ ID NO: 8.

Binding between the test compound and the dipeptidyl-peptidase IV-like enzyme polypeptide is detected. A test compound which binds to the dipeptidyl-peptidase IV-like enzyme polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the dipeptidyl-peptidase IV-like enzyme.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide, wherein the

polynucleotide comprises a nucleotide sequence selected from the group consisting of:
nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 7; and
the nucleotide sequence shown in SEQ ID NO: 7.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the dipeptidyl-peptidase IV-like enzyme through interacting with the dipeptidyl-peptidase IV-like enzyme mRNA.. ..

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a dipeptidyl-peptidase IV-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 55% identical to the amino acid sequence shown in SEQ ID NO: 8; and
the amino acid sequence shown in SEQ ID NO: 8.

A dipeptidyl-peptidase IV-like enzyme activity of the polypeptide is detected. A test compound which increases dipeptidyl-peptidase IV-like enzyme activity of the polypeptide relative to dipeptidyl-peptidase IV-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases dipeptidyl-peptidase IV-like enzyme activity of the polypeptide relative to dipeptidyl-peptidase IV-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a di-

peptidyl-peptidase IV-like enzyme product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:
nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 7; and
the nucleotide sequence shown in SEQ ID NO: 7.

Binding of the test compound to the dipeptidyl-peptidase IV-like enzyme product is detected. A test compound which binds to the dipeptidyl-peptidase IV-like enzyme product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:
nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 7; and
the nucleotide sequence shown in SEQ ID NO: 7.

Dipeptidyl-peptidase IV-like enzyme activity in the cell is thereby decreased.

The invention thus provides a human dipeptidyl-peptidase IV-like enzyme which can be used to identify test compounds which may act, for example, as agonists or antagonists at the enzyme's active site. Human dipeptidyl-peptidase IV-like enzyme and fragments thereof also are useful in raising specific antibodies which can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO: 1).

Fig. 2 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO:2).

Fig. 3 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO: 3).

Fig. 4 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO: 4).

Fig. 5 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO: 5).

Fig. 6 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO:6).

Fig. 7 shows the DNA-sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO: 7).

Fig. 8 shows the amino acid sequence deduced from the DNA-sequence of Fig. 7 (SEQ ID NO: 8).

Fig. 9 shows the amino acid sequence of the protein identified with SwissProt Accession No. P42658 (SEQ ID NO:9).

Fig. 10 shows the amino acid sequence of Pfam Accession No. PF01738 hmm dienelactone hydrolase (DLH) family (SEQ ID NO:10).

Fig. 11 shows the BLASTP alignment of dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO:8) with the protein identified by SwissProt Accession No. P42658 (SEQ ID NO:9).

Fig. 12 shows the alignment of dipeptidyl-peptidase IV-like enzyme polypeptide (SEQ ID NO:8) with the Pfam Accession No. PF01738 (SEQ ID NO:10) hmm dienelactone hydrolase (DLH) family.

Fig. 13 shows the abundance of human dipeptidyl-peptidase IV-like enzyme in phage libraries

Fig. 14 shows the gene expression of human dipeptidyl-peptidase IV-like enzyme in a human organ panel

Fig. 15 shows the gene expression of human dipeptidyl-peptidase IV-like enzyme in a human cardiovascular disease (CV) panel

Fig. 16 shows the gene expression of human dipeptidyl-peptidase IV-like enzyme in a CNS panel

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 55% identical to
the amino acid sequence shown in SEQ ID NO: 8; and
the amino acid sequence shown in SEQ ID NO: 8.

b) a polynucleotide comprising the sequence of SEQ ID NO: 7;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to ~~the degeneration of the genetic~~ code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a novel dipeptidyl-peptidase IV-like enzyme, particularly a human dipeptidyl-peptidase IV-like enzyme, is a discovery of the present invention. Human dipeptidyl-peptidase IV-like enzyme comprises the amino acid sequence shown in SEQ ID NO:8, as encoded by the complement of the nucleotide sequence shown in SEQ ID NO:7. A number of ESTs are contained within the coding sequence of human

dipeptidyl-peptidase IV-like enzyme (SEQ ID NOS:1-6), indicating that the complement of SEQ ID NO:7 is expressed.

Human dipeptidyl-peptidase IV-like enzyme is 53% identical over a 114 amino acid overlap to the human protein identified by Swiss Prot Accession No. P42658 (SEQ ID NO:9) and annotated as dipeptidyl peptidase IV-like protein (FIG. 11). Human dipeptidyl-peptidase IV-like enzyme also contains many identities to amino acids present in a hidden Markov model (hmm) of dienelactone hydrolase domains derived from 42 dienelactone hydrolase-like sequences, as shown in FIG. 2.

The human dipeptidyl-peptidase IV-like enzyme of the invention is expected to be useful for the same purposes as previously identified dipeptidyl-peptidase IV enzymes. Thus, human dipeptidyl-peptidase IV-like enzyme can be used in therapeutic methods to treat disorders such as tumors and neurodegenerative diseases. Human dipeptidyl-peptidase IV-like enzyme also can be used to screen for human dipeptidyl-peptidase IV-like enzyme agonists and antagonists

Polypeptides

Dipeptidyl-peptidase IV-like enzyme polypeptides according to the invention comprise at least 14, 25, 50, 75, 100, 125, 150, 175, or 186 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:8 or a biologically active variant thereof, as defined below. A dipeptidyl-peptidase IV-like enzyme polypeptide of the invention therefore can be a portion of a dipeptidyl-peptidase IV-like enzyme, a full-length dipeptidyl-peptidase IV-like enzyme, or a fusion protein comprising all or a portion of a dipeptidyl-peptidase IV-like enzyme.

Biologically Active Variants

Dipeptidyl-peptidase IV-like enzyme polypeptide variants which are biologically active, *i.e.*, retain a dipeptidyl-peptidase IV-like activity, also are dipeptidyl-peptidase IV-like enzyme polypeptides. Preferably, naturally or non-naturally occurring dipeptidyl-peptidase IV-like enzyme polypeptide variants

have amino acid sequences which are at least about 55, 60, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, or 98% identical to the amino acid sequence shown in SEQ ID NO:8 or a fragment thereof. Percent identity between a putative dipeptidyl-peptidase IV-like enzyme polypeptide variant and an amino acid sequence of SEQ ID NO:8 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a dipeptidyl-peptidase IV-like enzyme polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active dipeptidyl-peptidase IV-like enzyme polypeptide can readily be determined by assaying for dipeptidyl-peptidase IV-like activity, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against dipeptidyl-peptidase IV-like enzyme polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a dipeptidyl-peptidase IV-like enzyme polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the

yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A dipeptidyl-peptidase IV-like enzyme polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment can comprise at least 14, 25, 50, 75, 100, 125, 150, or 175 contiguous amino acids of SEQ ID NO:8 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length dipeptidyl-peptidase IV-like enzyme protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the dipeptidyl-peptidase IV-like enzyme polypeptide-encoding sequence and the heterologous protein sequence, so that the dipeptidyl-peptidase IV-like enzyme polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NO:7 in proper reading frame with nucleotides encoding the second polypeptide segment

and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human dipeptidyl-peptidase IV-like enzyme polypeptide can be obtained using dipeptidyl-peptidase IV-like enzyme polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of dipeptidyl-peptidase IV-like enzyme polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A dipeptidyl-peptidase IV-like enzyme polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a dipeptidyl-peptidase IV-like enzyme polypeptide. The complement of a coding sequence for human dipeptidyl-peptidase IV-like enzyme is shown in SEQ ID NO:7.

Degenerate nucleotide sequences encoding human dipeptidyl-peptidase IV-like enzyme polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO:8 also are dipeptidyl-peptidase IV-like enzyme polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of dipeptidyl-peptidase IV-like enzyme

polynucleotides which encode biologically active dipeptidyl-peptidase IV-like enzyme polypeptides also are dipeptidyl-peptidase IV-like enzyme polynucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the dipeptidyl-peptidase IV-like enzyme polynucleotides described above also are dipeptidyl-peptidase IV-like enzyme polynucleotides. Typically, homologous dipeptidyl-peptidase IV-like enzyme polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known dipeptidyl-peptidase IV-like polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions: 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the dipeptidyl-peptidase IV-like enzyme polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of dipeptidyl-peptidase IV-like enzyme polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human dipeptidyl-peptidase IV-like enzyme polynucleotides or dipeptidyl-peptidase IV-like enzyme polynucleotides of other species can therefore be identified by hybridizing a putative homologous dipeptidyl-peptidase IV-like enzyme polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:7 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising

polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to dipeptidyl-peptidase IV-like enzyme polynucleotides or their complements following stringent hybridization and/or wash conditions also are dipeptidyl-peptidase IV-like enzyme polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a dipeptidyl-peptidase IV-like enzyme polynucleotide having a nucleotide sequence shown in SEQ ID NO:7 or the complement thereof and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5\ °C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l$,

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of Polynucleotides

A dipeptidyl-peptidase IV-like enzyme polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for

isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated dipeptidyl-peptidase IV-like enzyme polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises dipeptidyl-peptidase IV-like enzyme nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Dipeptidyl-peptidase IV-like enzyme cDNA molecules can be made with standard molecular biology techniques, using dipeptidyl-peptidase IV-like enzyme mRNA as a template. Dipeptidyl-peptidase IV-like enzyme cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize dipeptidyl-peptidase IV-like enzyme polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a dipeptidyl-peptidase IV-like enzyme polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:8 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are

transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 2230 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 30553060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' nontranscribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Dipeptidyl-peptidase IV-like enzyme polypeptides can be obtained, for example, by purification from human cells, by expression of dipeptidyl-peptidase IV-like enzyme polynucleotides, or by direct chemical synthesis.

Protein Purification

Dipeptidyl-peptidase IV-like enzyme polypeptides can be purified from any cell which expresses the enzyme, including host cells which have been transfected with dipeptidyl-peptidase IV-like enzyme expression constructs. Brain and multiple sclerosis lesions provide especially useful sources of dipeptidyl-peptidase IV-like enzyme polypeptides. A purified dipeptidyl-peptidase IV-like enzyme polypeptide is separated from other compounds which normally associate with the dipeptidyl-peptidase IV-like enzyme polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified dipeptidyl-peptidase IV-like enzyme polypeptides is at least 80% pure; preferably, the preparations are

90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

To express a dipeptidyl-peptidase IV-like enzyme polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding dipeptidyl-peptidase IV-like enzyme polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those nontranslated regions of the vector enhancers, promoters, 5' and 3' untranslated regions which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems,

inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the dipeptidyl-peptidase IV-like enzyme polypeptide. For example, when a large quantity of a dipeptidyl-peptidase IV-like enzyme polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the dipeptidyl-peptidase IV-like enzyme polypeptide can be ligated into the vector in frame with sequences for the amino terminal Met and the subsequent ~~7 residues of~~ β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 55035509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding dipeptidyl-peptidase IV-like enzyme polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 16711680, 1984; Broglie *et al.*, *Science 224*, 838843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191196, 1992).

An insect system also can be used to express a dipeptidyl-peptidase IV-like enzyme polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding dipeptidyl-peptidase IV-like enzyme polypeptides can be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of dipeptidyl-peptidase IV-like enzyme polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which dipeptidyl-peptidase IV-like enzyme polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 32243227, 1994).

Mammalian Expression Systems

A number of viral based expression systems can be used to express dipeptidyl-peptidase IV-like enzyme polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding dipeptidyl-peptidase IV-like enzyme polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a dipeptidyl-peptidase IV-like enzyme polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 36553659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding dipeptidyl-peptidase IV-like enzyme polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be

enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed dipeptidyl-peptidase IV-like enzyme polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Posttranslational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for Posttranslational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high yield production of recombinant proteins. For example, cell lines which stably express dipeptidyl-peptidase IV-like enzyme polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 12 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced dipeptidyl-peptidase IV-like enzyme sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 22332, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 81723, 1980) genes which can be employed in *tk*⁻ or *aprt*⁻ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 356770, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 114, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 804751, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the dipeptidyl-peptidase IV-like enzyme polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a dipeptidyl-peptidase IV-like enzyme polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the dipeptidyl-peptidase IV-like enzyme polynucleotide.

Alternatively, host cells which contain a dipeptidyl-peptidase IV-like enzyme polynucleotide and which express a dipeptidyl-peptidase IV-like enzyme polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a dipeptidyl-peptidase IV-like enzyme polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide to detect transformants which contain a dipeptidyl-peptidase IV-like enzyme polynucleotide.

A variety of protocols for detecting and measuring the expression of a dipeptidyl-peptidase IV-like enzyme polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A tweezed, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a dipeptidyl-peptidase IV-like enzyme polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 12111216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides-encoding dipeptidyl-peptidase IV-like enzyme polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled

nucleotide. Alternatively, sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode dipeptidyl-peptidase IV-like enzyme polypeptides can be designed to contain signal sequences which direct secretion of soluble dipeptidyl-peptidase IV-like enzyme polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound dipeptidyl-peptidase IV-like enzyme polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a dipeptidyl-peptidase IV-like enzyme polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as

those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the dipeptidyl-peptidase IV-like enzyme polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a dipeptidyl-peptidase IV-like enzyme polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263281, 1992), while the enterokinase cleavage site provides a means for purifying the dipeptidyl-peptidase IV-like enzyme polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441453, 1993.

Chemical Synthesis

Sequences encoding a dipeptidyl-peptidase IV-like enzyme polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225232, 1980). Alternatively, a dipeptidyl-peptidase IV-like enzyme polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 21492154, 1963; Roberge *et al.*, *Science 269*, 202204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of dipeptidyl-peptidase IV-like enzyme polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic dipeptidyl-peptidase IV-like enzyme polypeptide can be

confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the dipeptidyl-peptidase IV-like enzyme polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce dipeptidyl-peptidase IV-like polypeptide encoding nucleotide sequences possessing nonnatural occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter dipeptidyl-peptidase IV-like enzyme polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a dipeptidyl-peptidase IV-like enzyme polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a dipeptidyl-peptidase IV-like enzyme polypeptide. Typically, at least 6, 8, 10, or 12

contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a dipeptidyl-peptidase IV-like enzyme polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a dipeptidyl-peptidase IV-like enzyme polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to dipeptidyl-peptidase IV-like enzyme polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a dipeptidyl-peptidase IV-like enzyme polypeptide from solution.

Dipeptidyl-peptidase IV-like enzyme polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a dipeptidyl-peptidase IV-like enzyme polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants

used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a dipeptidyl-peptidase IV-like enzyme polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human Bell hybridoma technique, and the EBV HYBRIDOMA technique (Kohler *et al.*, *Nature 256*, 495497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 3142, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 20262030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 68516855, 1984; Neuberger *et al.*, *Nature 312*, 604608, 1984; Takeda *et al.*, *Nature 314*, 452454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a dipeptidyl-peptidase IV-like enzyme polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which

specifically bind to dipeptidyl-peptidase IV-like enzyme polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 1112023, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to dipeptidyl-peptidase IV-like enzyme polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 38333837, 1989; Winter *et al.*, *Nature 349*, 293299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and

which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a dipeptidyl-peptidase IV-like enzyme polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of dipeptidyl-peptidase IV-like enzyme gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, ~~or a~~ combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 18, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543583, 1990.

Modifications of dipeptidyl-peptidase IV-like enzyme gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the dipeptidyl-peptidase IV-like enzyme gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions 10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a dipeptidyl-peptidase IV-like enzyme polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a dipeptidyl-peptidase IV-like enzyme polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent dipeptidyl-peptidase IV-like enzyme nucleotides, can provide sufficient targeting specificity for dipeptidyl-peptidase IV-like enzyme mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular dipeptidyl-peptidase IV-like enzyme polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a dipeptidyl-peptidase IV-like enzyme polynucleotide. These

modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 35393542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 15321539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a dipeptidyl-peptidase IV-like enzyme polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the dipeptidyl-peptidase IV-like enzyme polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to

the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a dipeptidyl-peptidase IV-like enzyme RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate dipeptidyl-peptidase IV-like enzyme RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease dipeptidyl-peptidase IV-like enzyme expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of

regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a dipeptidyl-peptidase IV-like enzyme polypeptide or a dipeptidyl-peptidase IV-like enzyme polynucleotide. A test compound preferably binds to a dipeptidyl-peptidase IV-like enzyme polypeptide or polynucleotide. More preferably, a test compound decreases or increases dipeptidyl-peptidase IV-like activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.*

33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see*, *e.g.*, Houghten, *BioTechniques 13*, 412421, 1992), or on beads (Lam, *Nature 354*, 8284, 1991), chips (Fodor, *Nature 364*, 555556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 18651869, 1992), or phage (Scott & Smith, *Science 249*, 386390, 1990; Devlin, *Science 249*, 404406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 63786382, 1990; Felici, *J. Mol. Biol. 222*, 301310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to dipeptidyl-peptidase IV-like enzyme polypeptides or polynucleotides or to affect dipeptidyl-peptidase IV-like enzyme activity or dipeptidyl-peptidase IV-like enzyme gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, Free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 161418 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 710, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV LIGHT. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 5763 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies, for example, the ATP/GTP binding site of the enzyme or the active site of the dipeptidyl-peptidase IV-like enzyme polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide like molecules.

In binding assays, either the test compound or the dipeptidyl-peptidase IV-like enzyme polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase,

alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the dipeptidyl-peptidase IV-like enzyme polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a dipeptidyl-peptidase IV-like enzyme polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a dipeptidyl-peptidase IV-like enzyme polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a dipeptidyl-peptidase IV-like enzyme polypeptide (McConnell *et al.*, *Science 257*, 19061912, 1992).

Determining the ability of a test compound to bind to a dipeptidyl-peptidase IV-like enzyme polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 23382345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a dipeptidyl-peptidase IV-like enzyme polypeptide can be used as a "bait protein" in a two hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 1204612054, 1993; Bartel *et al.*, *BioTechniques 14*, 920924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 16931696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the dipeptidyl-peptidase IV-like enzyme polypeptide and modulate its activity.

The two hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA BINDING and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein dependent complex, the DNA BINDING and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the dipeptidyl-peptidase IV-like enzyme polypeptide.

It may be desirable to immobilize either the dipeptidyl-peptidase IV-like enzyme polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the dipeptidyl-peptidase IV-like enzyme polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the dipeptidyl-peptidase IV-like enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array,

so that the location of individual test compounds can be tracked. Binding of a test compound to a dipeptidyl-peptidase IV-like enzyme polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the dipeptidyl-peptidase IV-like enzyme polypeptide is a fusion protein comprising a domain that allows the dipeptidyl-peptidase IV-like enzyme polypeptide to be bound to a solid support. For example, glutathione S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the nonadsorbed dipeptidyl-peptidase IV-like enzyme polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a dipeptidyl-peptidase IV-like enzyme polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated dipeptidyl-peptidase IV-like enzyme polypeptides (or polynucleotides) or test compounds can be prepared from biotinNHS(Nhydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a dipeptidyl-peptidase IV-like enzyme polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the ATP/GTP binding site or the active site of the dipeptidyl-peptidase IV-like enzyme

polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-IMMOBILIZED complexes, include immunodetection of complexes using antibodies which specifically bind to the dipeptidyl-peptidase IV-like enzyme polypeptide or test compound, enzyme linked assays which rely on detecting an activity of the dipeptidyl-peptidase IV-like enzyme polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a dipeptidyl-peptidase IV-like enzyme polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a dipeptidyl-peptidase IV-like enzyme polypeptide or polynucleotide can be used in a cell-based assay system. A dipeptidyl-peptidase IV-like enzyme polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a dipeptidyl-peptidase IV-like enzyme polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the dipeptidyl-peptidase IV activity of a human dipeptidyl-peptidase IV-like enzyme polypeptide. Dipeptidyl-peptidase IV activity can be measured, for example, as described in U.S. Patent 5,601,986 (see Example 2).

Enzyme assays can be carried out after contacting either a purified dipeptidyl-peptidase IV-like enzyme polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a transketolase activity of a dipeptidyl-peptidase IV-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing dipeptidyl-peptidase IV-like enzyme

activity. A test compound which increases a transketolase activity of a human dipeptidyl-peptidase IV-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human dipeptidyl-peptidase IV-like enzyme activity.

Gene Expression

In another embodiment, test compounds which increase or decrease dipeptidyl-peptidase IV-like enzyme gene expression are identified. A dipeptidyl-peptidase IV-like enzyme polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the dipeptidyl-peptidase IV-like enzyme polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of dipeptidyl-peptidase IV-like enzyme mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a dipeptidyl-peptidase IV-like enzyme polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a dipeptidyl-peptidase IV-like enzyme polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a dipeptidyl-peptidase IV-like enzyme polynucleotide can be used in a cell-based assay system. The dipeptidyl-peptidase IV-like enzyme polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a dipeptidyl-peptidase IV-like enzyme polypeptide, dipeptidyl-peptidase IV-like enzyme polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a dipeptidyl-peptidase IV-like enzyme polypeptide, or mimetics, agonists, antagonists, or inhibitors of a dipeptidyl-peptidase IV-like enzyme polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for

oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethylcellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 150 mM histidine, 0.1%2% sucrose, and 27% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated

condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anticancer therapies rarely exceed 2.0.

The advent of genomics driven molecular target identification has opened up the possibility of identifying new cancer specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anticancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

The human dipeptidyl-peptidase IV-like gene provides a therapeutic target for preventing cancers and/or tumors associated with neurodegenerative diseases and nervous system tissues. Based on its immunoreactivity, the levels of dipeptidyl-peptidase IV enzyme are much higher in the immature central nervous system of fetuses and newborn children than in the adult human brain (3), indicating that dipeptidyl-peptidase IV is expressed in actively developing nervous tissue. However, the content of dipeptidyl-peptidase IV is higher in glioma and astrocytoma tissue compared to normal brain tissue (1), and is also abundant in an astrocytoma cell line (2). While dipeptidyl-peptidase IV activity is therefore a part of normal neural development, dipeptidyl-peptidase IV activity also is associated with the abnormal growth of neural tissue in gliomas and astrocytomas. Suppression of dipeptidyl-peptidase IV-like activity may therefore suppress growth of neural tumors, such as those that occur in gliomas and astrocytomas.

Dipeptidyl-peptidase IV activity has further been indicated as contributing to angiogenesis associated with neural tissues. The sympathetic neuro-cotransmitter neuropeptide Y has been evidenced to be angiogenic for human endothelial cells *in vitro*, as it promotes sprouting and adhesion of vessels, migration, proliferation, and capillary tube formation (4). Neuropeptide Y has also been shown to be angiogenic *in vivo* in a murine assay for angiogenesis (4). The angiogenic property of

neuropeptide Y appears to be mediated by the Y2 receptor, which recognizes neuropeptide Y after the terminal amino acids Tyr-Pro have been cleaved by dipeptidyl-peptidase IV. Based on the angiogenic properties of neuropeptide Y after cleavage by dipeptidyl-peptidase IV, neuropeptide Y has been implicated in angiogenesis that accompanies growth of neural tumors. Compounds directed against dipeptidyl-peptidase IV-like activity may therefore prove useful as therapeutics for inhibiting angiogenesis of neural tissue tumors. Neurodegenerative diseases, such as multiple sclerosis, also can be treated.

Central and peripheral nervous system disorders also can be treated, such as primary and secondary disorders after brain injury, disorders of mood, anxiety disorders, disorders of thought and volition, disorders of sleep and wakefulness, diseases of the motor unit, such as neurogenic and myopathic disorders, neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and processes of peripheral and chronic pain.

Pain that is associated with CNS disorders also can be treated by regulating the activity of human dipeptidyl-peptidase IV-like enzyme. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (*e.g.*, infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgiaradioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (*e.g.*, diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic

and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a dipeptidyl-peptidase IV-like enzyme polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above described screening assays for treatments as described herein.

A reagent which affects dipeptidyl-peptidase IV-like enzyme activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce dipeptidyl-peptidase IV-like enzyme activity. The reagent preferably binds to an expression product of a human dipeptidyl-peptidase IV-like enzyme gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of

targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques

are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases dipeptidyl-peptidase IV-like enzyme activity relative to the dipeptidyl-peptidase IV-like enzyme activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in

the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a dipeptidyl-peptidase IV-like enzyme gene or the activity of a dipeptidyl-peptidase IV-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a dipeptidyl-peptidase IV-like enzyme gene or the activity of a dipeptidyl-peptidase IV-like enzyme polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to dipeptidyl-peptidase IV-like enzyme-specific mRNA, quantitative RT-PCR, immunologic detection of a dipeptidyl-peptidase IV-like enzyme polypeptide, or measurement of dipeptidyl-peptidase IV-like enzyme activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human dipeptidyl-peptidase IV-like enzyme also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding dipeptidyl-peptidase IV-like enzyme in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 43974401, 1985). Thus, the detection of a specific DNA sequence can be performed

by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a dipeptidyl-peptidase IV-like enzyme also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of dipeptidyl-peptidase IV-like enzyme activity

The polynucleotide of SEQ ID NO: 7 is inserted into the expression vector pCEV4 and the expression vector pCEV4-dipeptidyl-peptidase IV-like enzyme polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and the dipeptidyl-peptidase IV-like enzyme activity is measured at 24 °C, by mixing 50 or 100 µl of cell extracts to 100 or 150 µl microliters of a reaction buffer containing 200 µM of chromogenic substrate, such as Gly-Pro-PNA (commercially available from Bachem, San Diego, Calif.) in 0.1 M Tris-HCl buffered Triton X-100 (0.1% v/v) at pH 7.0. The reactions are incubated for 30 minutes, and optical density readings are taken at 405 nm. During the reaction time course, several optical density readings are taken at different time points. Dipeptidyl-peptidase IV-like enzyme activity is expressed in nmol/min/ml based on the progression curve calculated from the concentration of hydrolyzed substrates. It is shown that SEQ ID NO: 8 has dipeptidyl-peptidase IV-like enzyme activity.

EXAMPLE 2

Expression of recombinant human dipeptidyl-peptidase IV-like enzyme

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human dipeptidyl-peptidase IV-like polypeptides in yeast. The dipeptidyl-peptidase IV-like enzyme-encoding DNA sequence is derived from the complement of SEQ ID NO:7. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5' end an initiation codon and at its 3' end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed

for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/mdHis6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (NiNTAResin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human dipeptidyl-peptidase IV-like enzyme polypeptide is obtained.

EXAMPLE 3

Identification of a test compound which decreases dipeptidyl-peptidase IV-like activity

Dipeptidyl-peptidase IV-like activity can be assayed using cellular extracts from human cell lines, such as the astrocytoma cell lines CRL-1718 or HTB-15. Test compounds from a small molecule library can be assayed for their ability to regulate dipeptidyl-peptidase IV-like activity by contacting CRL-1718 or HTB-15 cell line extracts with the test compounds. Control extracts, in the absence of a test compound, are also assayed. Dipeptidyl-peptidase IV-like activity can be assayed using a substrate which reacts with dipeptidyl-peptidase IV to form a detectable product, as described in U.S. Patent 5,601,986.

Suitable enzyme substrates include, but are not limited to, dipeptide substrates such as Xaa-pro-para-nitro-analide (Xaa-Pro-PNA) or Xaa-Pro-coumarin. The variable amino acid, Xaa, can be any naturally occurring or synthetic amino acid. An exemplary dipeptide substrate is Gly-Pro-para-nitro-analide (Gly-Pro-PNA). At a wavelength of 405 nanometers, the substrate has no absorbance; however, if the dipeptide substrate is cleaved (after the Pro) due to the presence of dipeptidyl-peptidase IV, the formation of a reaction product can be visualized

spectrophotometrically, as a yellow-green color is produced. Other substrates, such as Xaa-Pro-coumarin, can be visualized spectrofluorometrically as a fluorescent emission is produced by the reaction.

Dipeptidyl-peptidase IV Assays embodying such reagents and reactions can be performed in any suitable reaction vessel, for example, a test tube or well of a microtiter plate. Enzyme activities typically are measured at 24 °C, by mixing 50 or 100 µl of enzyme sample to 100 or 150 µl microliters of a reaction buffer containing 200 µM of chromogenic substrate, such as Gly-Pro-PNA (commercially available from Bachem, San Diego, Calif.) in 0.1 M Tris-HCl buffered Triton X-100 (0.1% v/v) at pH 7.0. The reactions are incubated for 30 minutes, and optical density readings are taken at 405 nm. During the reaction time course, several optical density readings are taken at different time points. Dipeptidyl-peptidase IV-like enzyme activity is expressed in nmol/min/ml based on the progression curve calculated from the concentration of hydrolyzed substrates.

A test compound which decreases dipeptidyl-peptidase IV-like activity of an enzyme relative to the control preparation by at least 20% is identified as a di-peptidyl-peptidase IV-like enzyme inhibitor.

EXAMPLE 4

Identification of test compounds that bind to dipeptidyl-peptidase IV-like enzyme polypeptides

Purified dipeptidyl-peptidase IV-like enzyme polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Dipeptidyl-peptidase IV-like enzyme polypeptides comprise the amino acid sequence shown in SEQ ID NO:8. The test compounds comprise a fluorescent tag. The samples are incubated for 5

minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a dipeptidyl-peptidase IV-like enzyme polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a dipeptidyl-peptidase IV-like enzyme polypeptide.

EXAMPLE 5

Identification of a test compound which decreases dipeptidyl-peptidase IV-like enzyme gene expression

A test compound is administered to a culture of human cells transfected with a dipeptidyl-peptidase IV-like enzyme expression construct and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 Fg total RNA and hybridized with a ^{32}P-labeled dipeptidyl-peptidase IV-like enzyme-specific probe at 65 ° C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from SEQ ID NO:7. A test compound which decreases the dipeptidyl-peptidase IV-like enzyme-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of dipeptidyl-peptidase IV-like enzyme gene expression.

EXAMPLE 6

Treatment of multiple sclerosis with a reagent which specifically binds to a dipeptidyl-peptidase IV-like enzyme gene product

Synthesis of antisense dipeptidyl-peptidase IV-like enzyme oligonucleotides comprising at least 11 contiguous nucleotides selected from SEQ ID NO:7 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al., Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361362, 1953).

The antisense oligonucleotides are administered to a patient with multiple sclerosis. The severity of the patient's multiple sclerosis is decreased.

EXAMPLE 7

Tissue expression of human dipeptidyl-peptidase IV-like enzyme

a) PCR analysis using cDNA phage libraries from human tissues

Human cDNA phage libraries (Stratagene) were ordered in a "human tissue panel IA" (unless differently specified) as described in Tab.1.

0.5 µl of each library purchased sample were used as template in PCR analysis regardless the title (phage/ml) for non quantitative expression analysis.

In addition a positive control PCR reaction was performed with about 20 ng of human genomic DNA as template and a negative control was performed with no template.

Standard PCR procedure were as indicated by Perkin Elmer.

PCR protocol was as follows:

Primers:

Primer A: 5'-GGATGCCATCTAAGGAAGAAAGCAC-3'

Primer B: 5'-CAGAACAAACAGGGGGATAACAGAAG-3'

PCR reaction mix:

0.5µl	template
1 x	Gold PCR Buffer (Perkin Elmer)
0.2 mM	dNTPs (Pharmacia)
1.5mM	MgCl2 (Perkin Elmer)
0.5 µM	primer A
0.5 µM	primer B
2.5 U	AmpliTaq Gold DNA Polymerase (Perkin Elmer)

to 25 µl final reaction volume with sterile H_2O.

Amplification protocol performed in Perkin Elmer 9700 thermocycler:

1 time the following step:

pre PCR 9' at 94° C

40 times the following steps:

denaturation 30'' at 94°C

annealing 1' at 63°C

elongation 30'' at 72°C

Expected length of specific PCR product: 618bp

Amplification products were analysed by electrophoresis on 2% agarose (SeaKem LE agarose, FMC bioproducts) gel in 1XTAE running buffer following standard procedure, as described by Maniatis et al.

PCR amplification products of the expected size were detectable some of the phage libraries.

The results are shown in Fig. 13.

Tab.1

Library	Description	Catalogue
Brain(corpus striatum)	Caudate and putamen, males, 57 & 63 years old	936213
Brain (foetal)	Male and female, Caucasian	937227
Brain (frontal cortex)	Female, 85 years old	936212
Brain (substantia nigra)	Male and female, 60 years old	936210
Brain (occipital cortex)	Female, 85 years old	936211
Brain stem	Female,2 years old	935206
Bronchial muscle	Human bronchial/tracheal smooth muscle primary cells	780032
Coronary	Coronary artery endothelial primary cells	780025

Library	Description	Catalogue
Coronary	Coronary artery smooth muscle primary cells	780029
Endothelial	Microvascular endothelial primary cells	780028
Heart	12 pooled, 19-50 years old, male/female Caucasian	937257
Kidney	8 pooled, whole kidney from 24-55 years old, male/female, Caucasian	937250
Liver	Normal,38 years old, Caucasian	937241
Lung	Male, 72 years old, normal	937210
Muscle (skeletal)	Female, 19 years old	936215
Ovary	Normal, 49 years old, Caucasian	937217
Pulmonary artery endothelial	Pulmonary artery endothelial primary cells	780027
Umbilical artery endothelial cells	Umbilical artery endothelial cells	780023
Whole Brain	Whole brain 60 year	HL5018t Clontech
Spinal chord	Whole, pooled from 26 male/female, 16-75 years	HL5001b Clontech
Aorta	Whole thoracic (intima & media) pooled from 1female/3male	HL1136a Clontech
Bone Marrow	Pooled from 51 male/female	HL5034t Clontech
Lymph Node	Whole lymph nodes pooled 34 male/female	HL5036t Clontech
Foetal spleen	Pooled from 6 spleens (13-22 weeks gestation)	937262
DRG		11137-015 Life Technologies

Quantitative analysis of relative expression of human dipeptidyl-peptidase IV-like enzyme in human tissues

Quantitative expression profiling was performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi et al., 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland et al.). Since the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid et al., 1996, and Gibson et al., 1996).

The amplification of an endogenous control can be performed to standardise the amount of sample RNA added to a reaction. In this kind of experiments the control of choice is the 18S ribosomal RNA. Since reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labelled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700 Sequence detector System (PE Applied Biosystems, Foster City, CA).

References

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- Holland, P.M., Abramson, R.D., Watson, R. and Gelfand, D.H. 1991. Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of Thermus aquaticus DNA polymerase. *Proc.Natl.Acad.Sci.* 88:7276-7280.
- Heid, C., Stevens, J., Livak, K. And Williams, P.M. 1996. Real time quantitative PCR. *Genome Res.* 6:986-994.
- Gibson, U.E., Heid, C.A. and Williams, P.M. 1996. A novel method for real time quantitative RT-PCR. *Genome Res.* 6: 995-1001.

cDNA preparation

The total RNAs used for expression quantification are listed in Table 1 along with their purchasers.

Fifty µgs of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix:

DNase I, RNase-free (Roche Diagnostics, Germany)	0.2 U/µL
Rnase inhibitor (PE Applied Biosystems, CA)	0.4 U/µL
Tris-HCl pH 7.9	10 mM
$MgCl_2$	10 mM
NaCl	50 mM
DTT	1 mM

After incubation, RNA was extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of NaAcetate 3M pH5.2 and 2 volumes ethanol.

After spectrophotometric quantification, each sample has been reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) accordingly to purchaser protocol. RNA final concentration in the reaction mix was 200ng/μL. Reverse transcription was made with 2.5μM of random hexamers.

TaqMan quantitative analysis

Specific primers and probe were designed accordingly to PE Applied Biosystems recommendations and are listed below:
forward primer: 5'-TTTTTAAATGTGGATCCGTGGTT-3'
reverse primer: 5'-GCATCCCAAGGTATCTTTCAGAGA-3'
probe: 5'-(FAM) CACCTATCACAGACTTGAAATTGTATGCCTCAGC (TAMRA)-3'
where FAM = 6-carboxy-fluorescein
and TAMRA = 6-carboxy-tetramethyl-rhodamine.
The expected length of the PCR product was 80bp.

Quantification experiments were performed on 50 ng of reverse transcribed RNA from each sample. Each determination was done in triplicate.

Total cDNA content was normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA by use of the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

Assay reaction mix was as follows:

	final
TaqMan Universal PCR Master Mix (2x) (PE Applied Biosystems, CA)	1x
PDAR control – 18S RNA (20x)	1x
Forward primer	900nM
Reverse primer	900nM
Probe	200nM
cDNA	10ng
Water	to 25µL

PCR conditions were:

1 time the following steps:

pre PCR	2' at 50°C
1	0' at 95°C

40 times the following steps:

denaturation	15'' at 95°C
annealing/extension	1' at 60°C

The experiment was performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

The results are shown in Figs.14 to 16.

RNA	Purch. & catalogue #
h. Fetal Brain	Clontech (CA) 640191
h. Brain	OriGene (MD) HT1001
h. Muscle	OriGene (MD) HT1008
h. Heart	OriGene (MD) HT1002
h. Lung	OriGene (MD) HT1009
h. Kidney	OriGene (MD) HT1003
h. Liver	OriGene (MD) HT1005
h. Thymus	Clontech (CA) 640281
h. Testis	OriGene (MD) HT1011
h. Colon	OriGene (MD) HT1015
h. Placenta	OriGene (MD) HT1013
h. Trachea	Clontech 640911
h. Pancreas	Clontech 640311
h. Gastric mucose	from autopsy
h. Foetal Liver	Clontech (CA) 640181
h. Bladder	Invitrogen (CA) D602001
h. Adrenal gland	Clontech (CA) 640161
h. Spleen	OriGene (MD) HT1004
h. Prostate	Clontech (CA) 640381
h. Prostate	from autopsy
h. hypertrophic prostate	from autopsy
h. brain	from autopsy
h. Cortex	from autopsy
h. Choroid plexus	from autopsy

RNA	Purch. & catalogue #
h. Hippocampus	from autopsy
h. Hypothalamus	from autopsy
h. Amygdala	from autopsy
h. Thalamus	from autopsy
h. Cerebellum	from autopsy
h. Cerebellum	Clontech (CA) 640351
h. Spinal Cord	Clontech (CA) K40031

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5. Pangalos *et al.*, (1999) Isolation and expression of novel human glutamate carboxypeptidases with N-acetylated alpha-linked acidic dipeptidase and dipeptidyl peptidase IV activity. *J Biol Chem* 274(13):8470-83.

CLAIMS

1. An isolated polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide and being selected from the group consisting of:

   a) a polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide comprising an amino acid sequence selected form the group consisting of:

   amino acid sequences which are at least about 55% identical to
   the amino acid sequence shown in SEQ ID NO: 8; and
   the amino acid sequence shown in SEQ ID NO: 8.

   b) a polynucleotide comprising the sequence of SEQ ID NO: 7;

   c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

   d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

   e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified dipeptidyl-peptidase IV-like enzyme polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a dipeptidyl-peptidase IV-like enzyme polypeptide, wherein the method comprises the following steps:

   a) culturing the host cell of claim 3 under conditions suitable for the expression of the dipeptidyl-peptidase IV-like enzyme polypeptide; and

b) recovering the dipeptidyl-peptidase IV-like enzyme polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a dipeptidyl-peptidase IV-like enzyme polypeptide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a dipeptidyl-peptidase IV-like enzyme polypeptide of claim 4 comprising the steps of: contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the dipeptidyl-peptidase IV-like enzyme polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a test compound with any dipeptidyl-peptidase IV-like enzyme polypeptide encoded by any polynucleotide of claim1;
detecting binding of the test compound to the dipeptidyl-peptidase IV-like enzyme polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a dipeptidyl-peptidase IV-like enzyme.

11. A method of screening for agents which regulate the activity of a dipeptidyl-peptidase IV-like enzyme, comprising the steps of:

contacting a test compound with a dipeptidyl-peptidase IV-like enzyme polypeptide encoded by any polynucleotide of claim 1; and
detecting a dipeptidyl-peptidase IV-like enzyme activity of the polypeptide, wherein a test compound which increases the dipeptidyl-peptidase IV-like enzyme activity is identified as a potential therapeutic agent for increasing the activity of the dipeptidyl-peptidase IV-like enzyme, and wherein a test compound which decreases the dipeptidyl-peptidase IV-like enzyme activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the dipeptidyl-peptidase IV-like enzyme.

12. A method of screening for agents which decrease the activity of a dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of dipeptidyl-peptidase IV-like enzyme.

13. A method of reducing the activity of dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any dipeptidyl-peptidase IV-like enzyme polypeptide of claim 4, whereby the activity of dipeptidyl-peptidase IV-like enzyme is reduced.

14. A reagent that modulates the activity of a dipeptidyl-peptidase IV-like enzyme polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a dipeptidyl-peptidase IV-like enzyme in a disease.

17. Use of claim 16 wherein the disease is a tumor or a peripheral or central nervous system disorder including pain or a neurodegenerative disorder.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8.

19. The cDNA of claim 18 which comprises SEQ ID NO: 7.

20. The cDNA of claim 18 which consists of SEQ ID NO: 7.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO: 7.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO: 7.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO: 8.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO: 8.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO: 7.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8, comprising the steps of:
hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 7 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 7; and
instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8, comprising:
an antibody which specifically binds to the polypeptide; and
instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 55% identical to the amino acid sequence shown in SEQ ID NO: 8 and (2) the amino acid sequence shown in SEQ ID NO: 8; and
detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human dipeptidyl-peptidase IV-like enzyme.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 55% identical to the amino acid sequence shown in SEQ ID NO: 8 and (2) the amino acid sequence shown in SEQ ID NO: 8; and
detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human dipeptidyl-peptidase IV-like enzyme, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human dipeptidyl-peptidase IV-like enzyme.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human dipeptidyl-peptidase IV-like enzyme, comprising the steps of:
contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO: 7; and
detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human dipeptidyl-peptidase IV-like enzyme.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human dipeptidyl-peptidase IV-like enzyme, comprising the step of:
contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 7, whereby the activity of a human dipeptidyl-peptidase IV-like enzyme is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:
a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8; and
a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 7; and
a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:
an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8; and
a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO: 7.

68. A method of treating a dipeptidyl-peptidase IV-like enzyme dysfunction related disease, wherein the disease is selected from a tumor or a peripheral or central nervous system disorder including pain or a neurodegenerative disorder, comprising the step of:
administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human dipeptidyl-peptidase IV-like enzyme, whereby symptoms of the dipeptidyl-peptidase IV-like enzyme dysfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

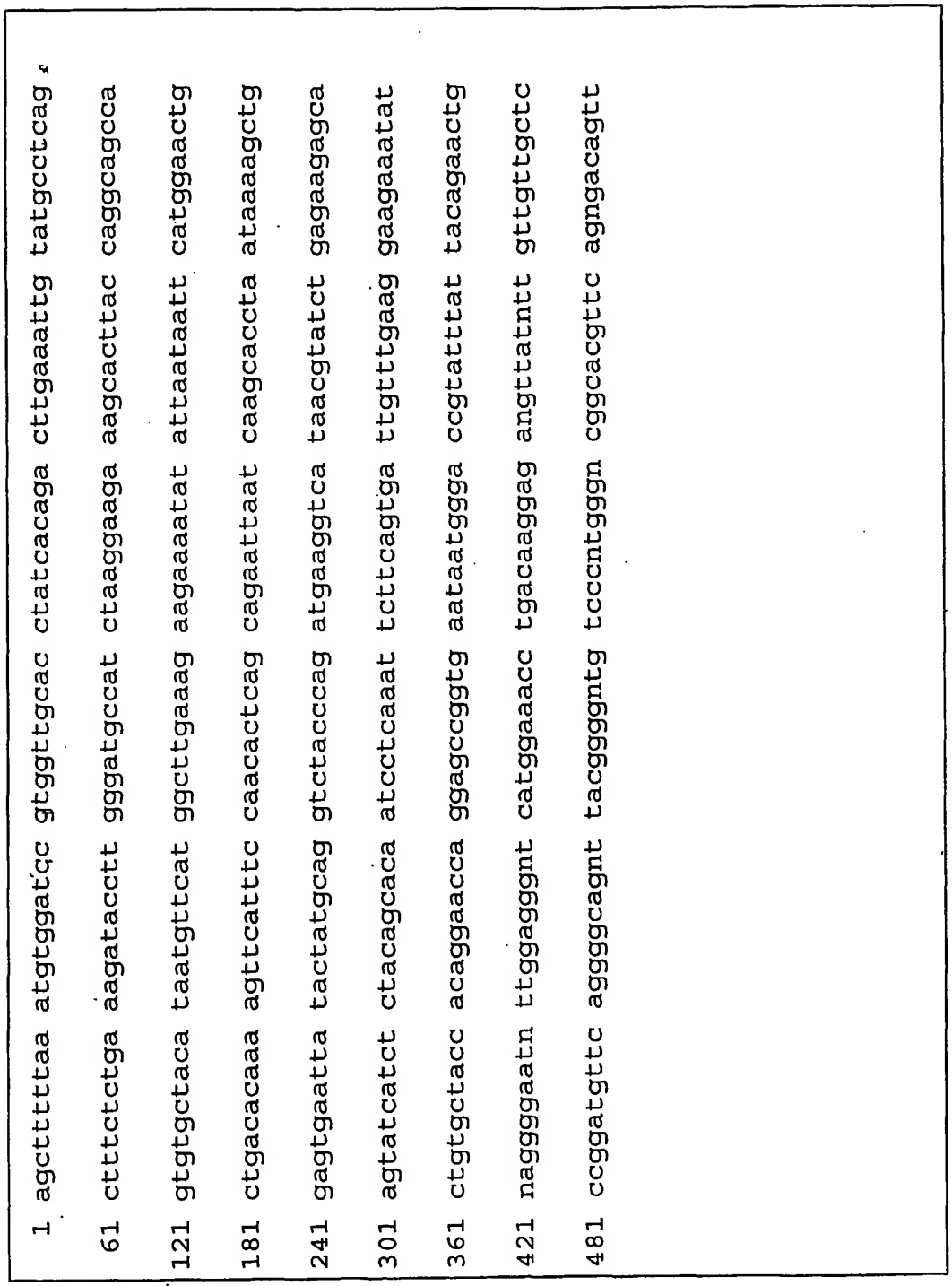
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Fig. 2

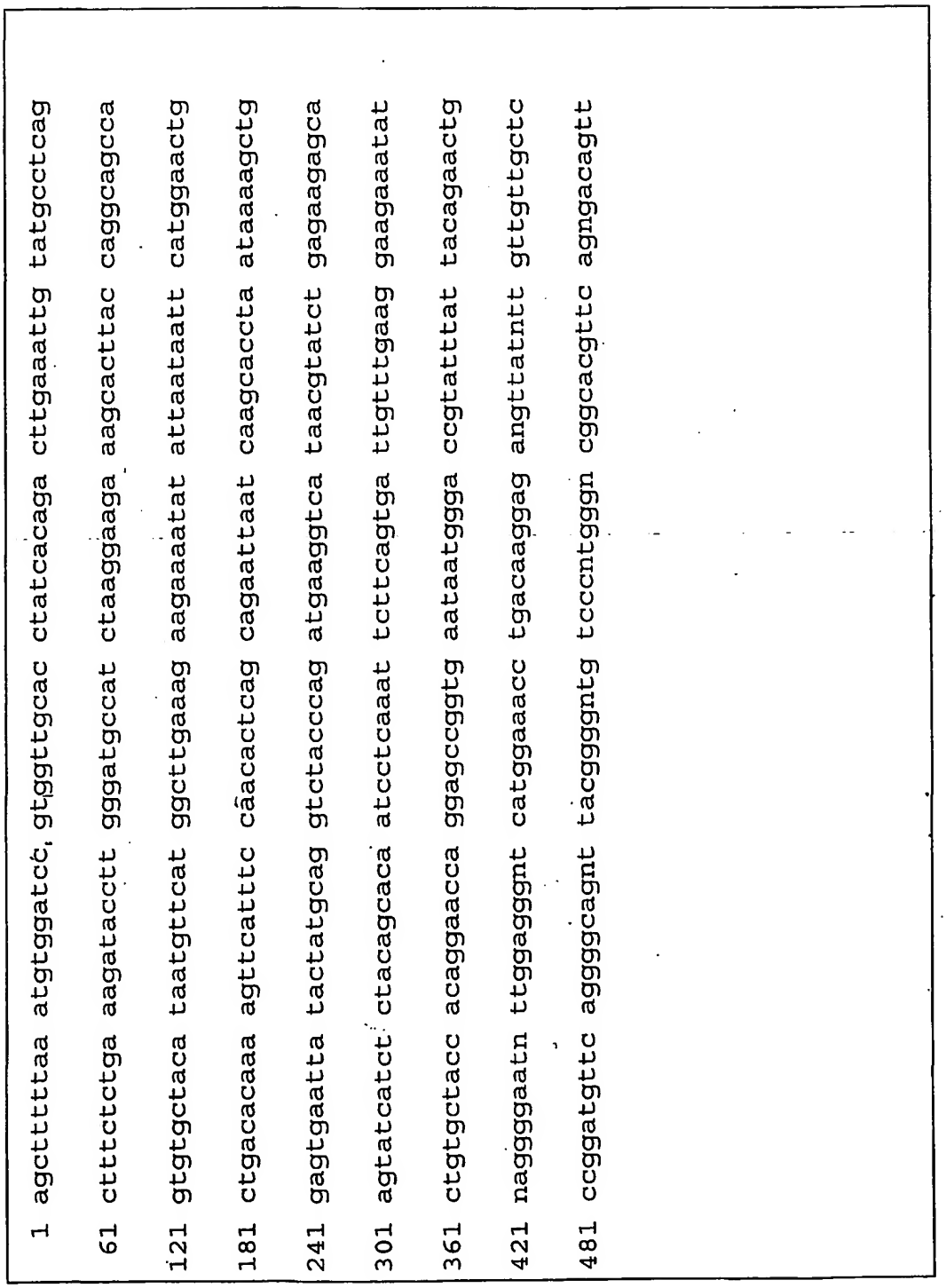
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Fig. 3

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Fig. 4

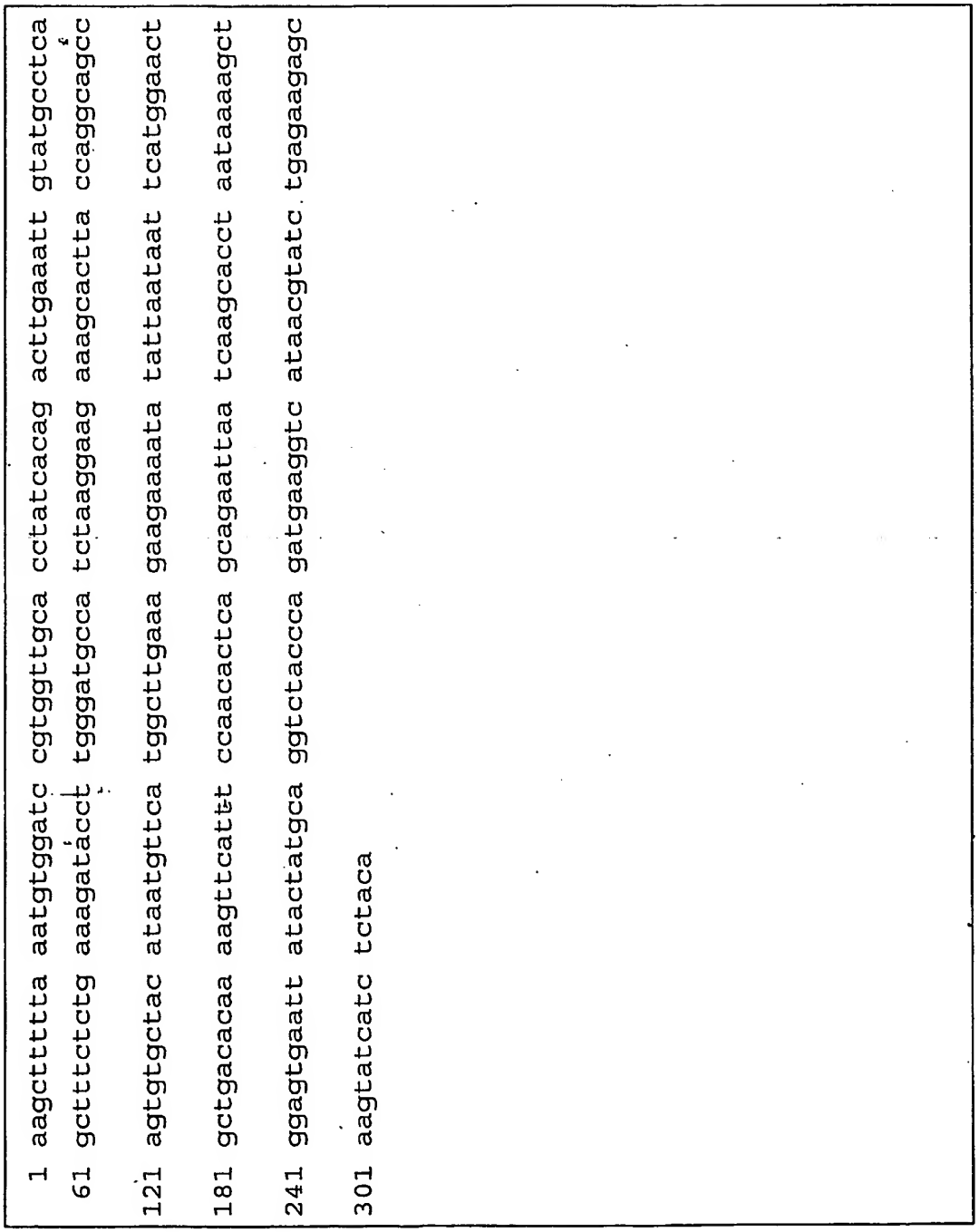
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Fig. 5

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Fig. 6

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Fig. 7

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Fig. 8

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APGCSGAXYG XVPWXGTFXD S

Fig. 9

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Fig. 10

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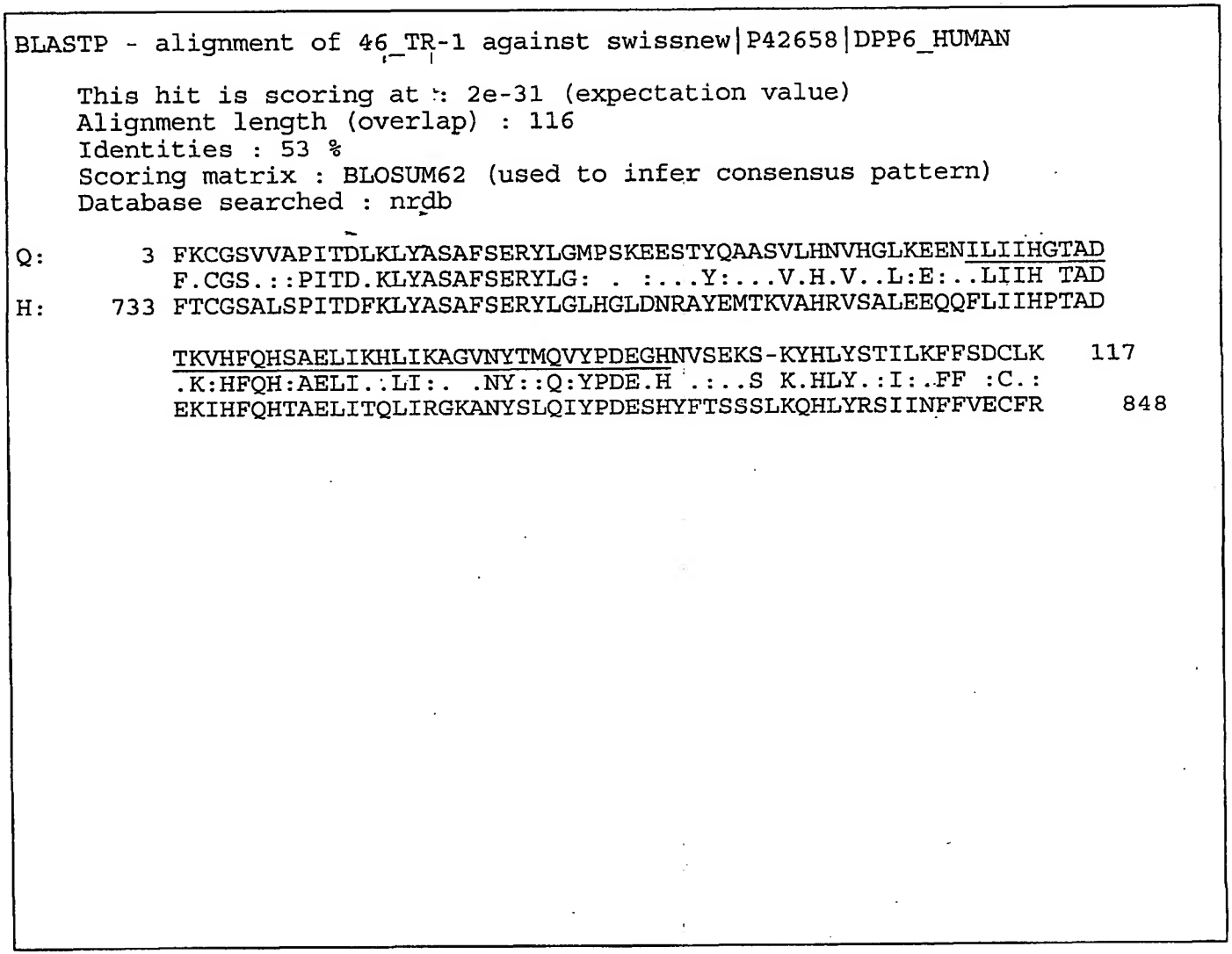
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H: 733 FTCGSALSPITDFKLYASAFSERYLGLHGLDNRAYEMTKVAHRVSALEEQQFLIIHPTAD
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Fig. 11

FIG. 12

```
HMMPFAM - alignment of 46_TR-1 against pfam|hmm|DLH
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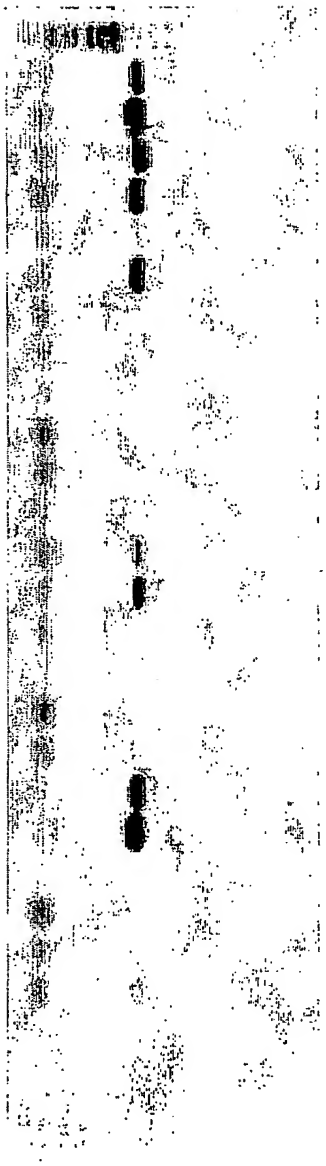

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6-Brain Stem
7-Bronchial muscle
8-Coronary (endot.cells)
9-Coronary (sm.muscle cells)
10-Endothelial
11-Hert
12-Kidney
13-Liver
14-Lung
15-Sk Muscle
16-Ovary
17-Pulmunary artery
18-Umbelical artery
19-Whole brain
20-Spinal cord
21-Aortha
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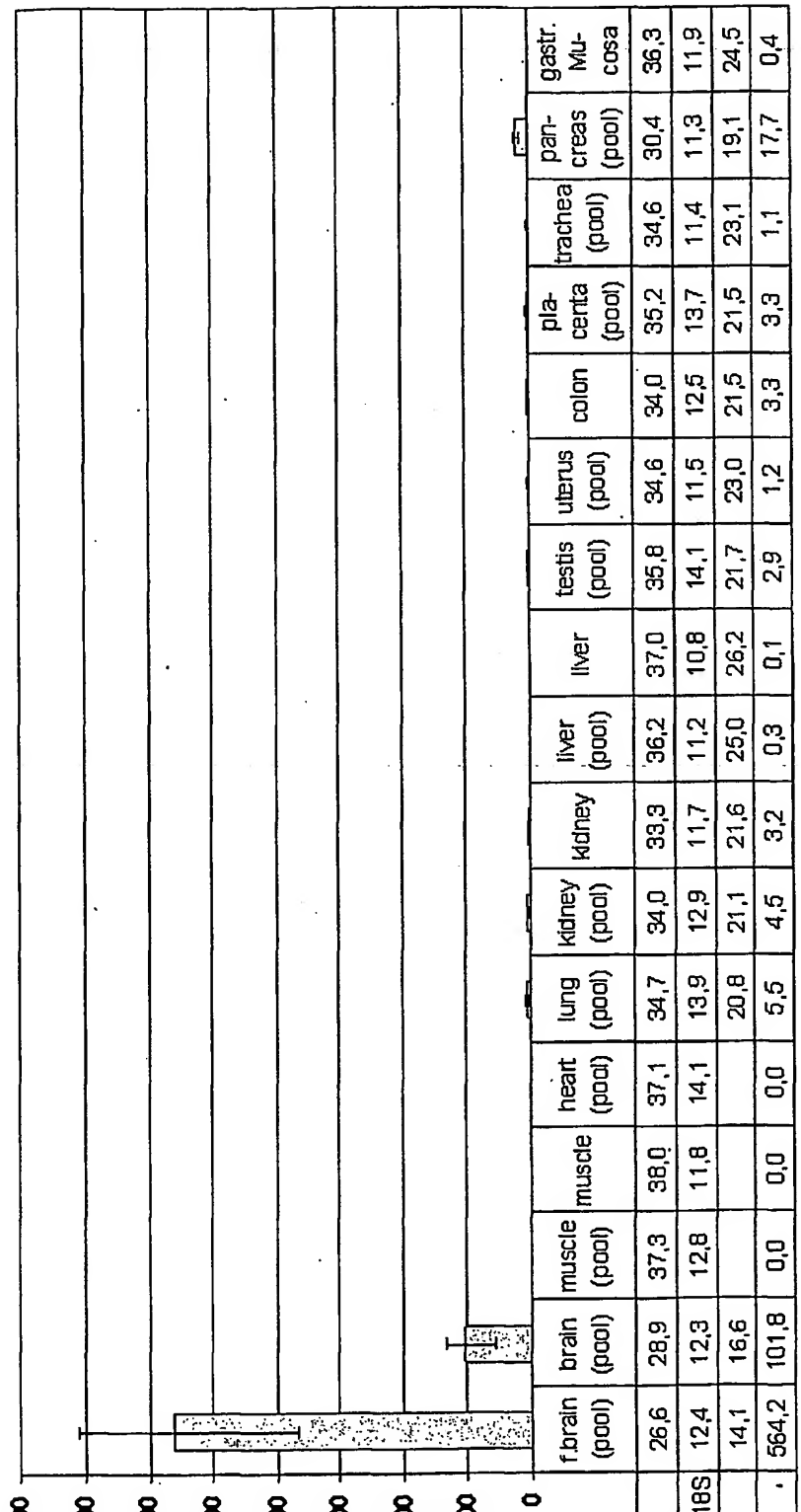
FIG. 14



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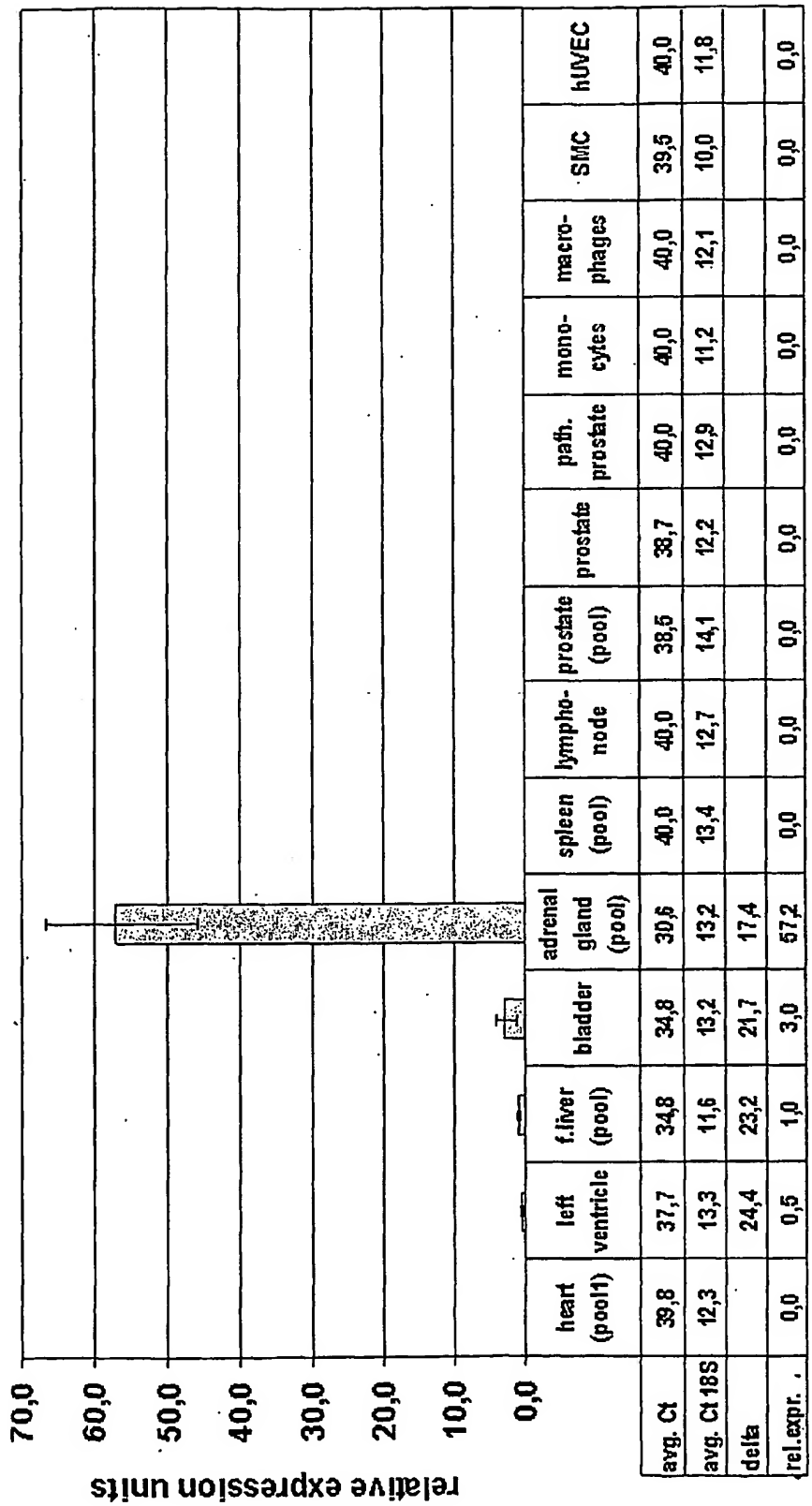
avg. Ct	26,6	28,9	37,3	38,0	37,1	34,7	34,0	33,3	36,2	37,0	35,8	34,6	34,0	35,2	34,6	30,4	36,3
avg. Ct 18S	12,4	12,3	12,8	11,8	14,1	13,9	12,9	11,7	11,2	10,8	14,1	11,5	12,5	13,7	11,4	11,3	11,9
delta	14,1	16,6				20,8	21,1	21,6	25,0	26,2	21,7	23,0	21,5	21,5	23,1	19,1	24,5
rel.expr.	564,2	101,8	0,0	0,0	0,0	5,5	4,5	3,2	0,3	0,1	2,9	1,2	3,3	3,3	1,1	17,7	0,4

FIG. 15



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avg. Ct 18S	12,3	13,3	11,6	13,2	13,2	13,4	12,7	14,1	12,2	12,9	11,2	12,1	10,0	11,8
delta		24,4	23,2	21,7	17,4									
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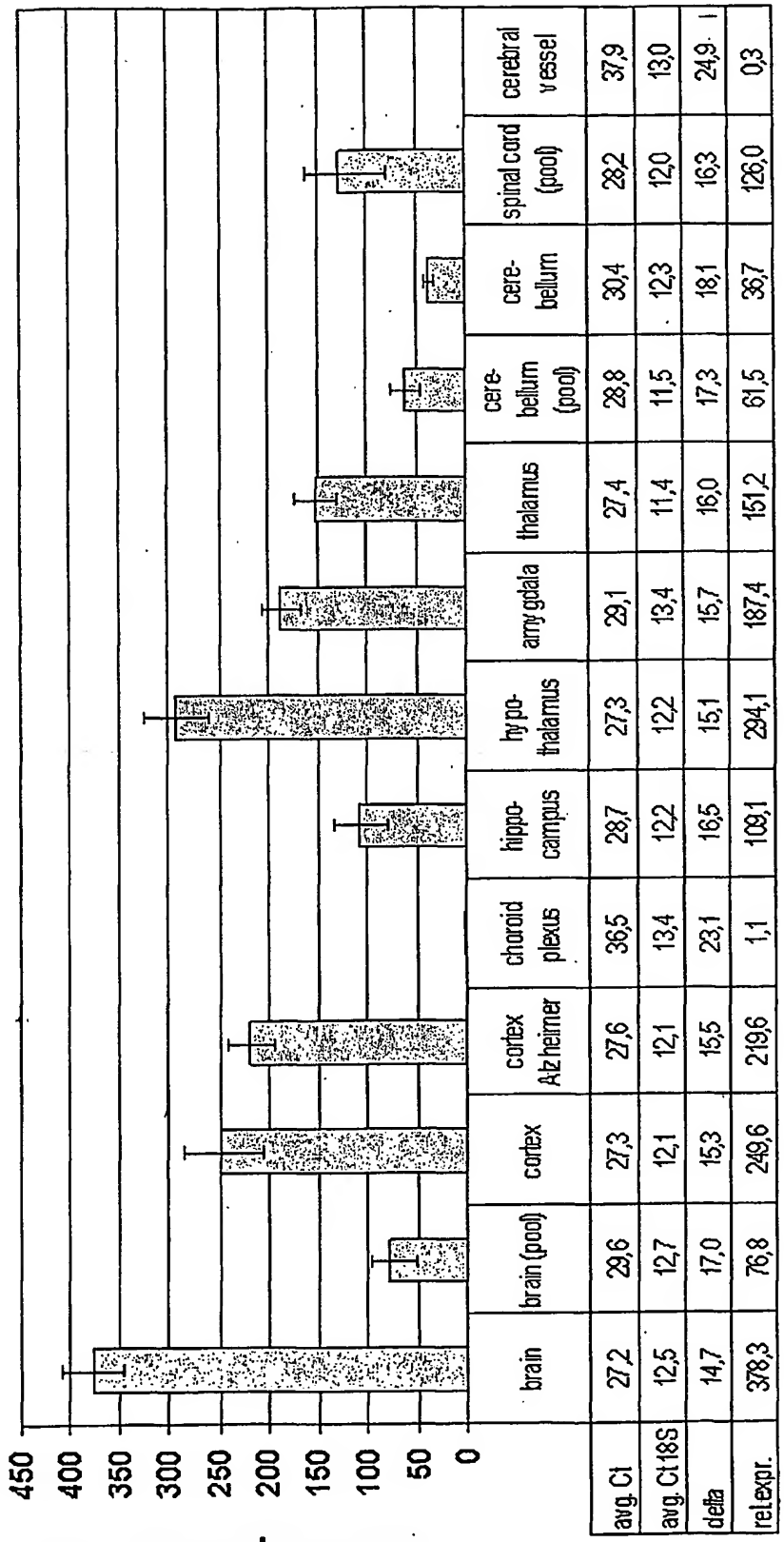

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avg. Ct 18S	12,5	12,7	12,1	12,1	13,4	12,2	12,2	13,4	11,4	11,5	12,3	12,0	13,0
delta	14,7	17,0	15,3	15,5	23,1	16,5	15,1	15,7	16,0	17,3	18,1	16,3	24,9
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FIG. 16

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gctttctctg aaagatacct tgggatgcca tctaaggaag aaagcactta ccaggcagcc 120

agtgtgctac ataatgttca tggcttgaaa gaagaaaata tattaataat tcatggaact 180

gctgacacaa aagttcattt ccaacactca gcagaattaa tcaagcacct aataaaagct 240

ggagtgaatt atactatgca ggtctaccca gatgaaggtc ataacgtatc tgagaagagc 300

aagtatcatc tctaca 316

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<212> DNA
<213> Homo sapiens

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gaactgctga cacaaaagtt catttccaac actcagcaga attaatcaag cacctaataa 120

aagctggagt gaattatact atgcaggtct acccagatga aggtcataac gtatctgaga 180

agagcaagta tcatctctac agcacaatcc tcaaattctt cagtgattgt ttgaagggag 240

gaatatctgt gctaccac 258

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aaagctggag tgaattatac tatgcaggtc tacccagatg aaggtcataa cgtatctgag 120

aagagcaagt atcatctcta cagcacaatc ctcaaattct ncagtgattg tttgnnggaa 180

gaaatatctg tgctaccaca ggaaccagaa gaagatgaat aatggaccgt atttatacag 240

aactgaaggg aatattgagg ctcaatgaaa cctgacaaag agactgtnat attgtagttg 300

ctccagaatg tcaagggcag cttacggnga atgtcactgg nagcagcacg ctcnagaggn 360

cagtgaactt ngcaatttt 379

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ccagttctgt aataaatacg gtcccattat tccaccggcc tcctggttcc tgtggtagca 180

cagatatttc ttccttcaaa caatcactga agaatttgag gattgtgctg tagagatgat 240

acttgctctt ctcagatacg ttatgacctt catctgggta gacctgcata gtataattca 300

ctccagcttt tattaggtgc ttgattaatt ctgctgagtg ttggaaatga acttttgtgt 360

cagcagttcc atgaattatt aatatatttt cttctttcaa gccatgaaca ttatgtagca 420

cactggctgc ctggtaagtg ctttcttcct tagatggcat cccaaggtat ctttcagaga 480

aagctgaggc atacaatttc aagtctgtga taggtgcaac cacggatcca catttaaaaa 540

gctt 544

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Leu Tyr Ala Ser Ala Phe Ser Glu Arg Tyr Leu Gly Met Pro Ser Lys
            20                  25                  30

Glu Glu Ser Thr Tyr Gln Ala Ala Ser Val Leu His Asn Val His Gly
        35                  40                  45

Leu Lys Glu Glu Asn Ile Leu Ile Ile His Gly Thr Ala Asp Thr Lys
    50                  55                  60

Val His Phe Gln His Ser Ala Glu Leu Ile Lys His Leu Ile Lys Ala
65                  70                  75                  80

Gly Val Asn Tyr Thr Met Gln Val Tyr Pro Asp Glu Gly His Asn Val
                85                  90                  95

Ser Glu Lys Ser Lys Tyr His Leu Tyr Ser Thr Ile Leu Lys Phe Phe
            100                 105                 110

Ser Asp Cys Leu Lys Glu Glu Ile Ser Val Leu Pro Gln Glu Pro Gly
        115                 120                 125

Gly Arg Trp Asn Asn Gly Thr Val Phe Ile Thr Glu Leu Glu Gly Glu
    130                 135                 140

Tyr Trp Arg Val His Gly Asn Leu Thr Arg Arg Xaa Tyr Xaa Val Val
145                 150                 155                 160

Ala Pro Gly Cys Ser Gly Ala Xaa Tyr Gly Xaa Val Pro Trp Xaa Gly
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Thr Phe Xaa Asp Ser
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<213> Homo sapiens

<400> 9

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Met Ala Ser Leu Tyr Gln Arg Phe Thr Gly Lys Ile Asn Thr Ser Arg
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Ser Phe Pro Ala Pro Pro Glu Ala Ser His Leu Leu Gly Gly Gln Gly
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Pro Glu Glu Asp Gly Gly Ala Gly Ala Lys Pro Leu Gly Pro Arg Ala
        35                  40                  45

Gln Ala Ala Ala Pro Arg Glu Arg Gly Gly Gly Gly Gly Gly Ala Gly
    50                  55                  60

Gly Arg Pro Arg Phe Gln Tyr Gln Gly Arg Ser Asp Gly Asp Glu Glu
65                  70                  75                  80

Asp Glu Leu Val Gly Ser Asn Pro Pro Gln Arg Asn Trp Lys Gly Ile
                85                  90                  95

Ala Ile Ala Leu Leu Val Ile Leu Val Ile Cys Ser Leu Ile Val Thr
            100                 105                 110

Ser Val Ile Leu Leu Thr Pro Ala Glu Asp Asn Ser Leu Ser Gln Lys
        115                 120                 125

Lys Lys Val Thr Val Glu Asp Leu Phe Ser Glu Asp Phe Lys Ile His
    130                 135                 140

Asp Pro Glu Ala Lys Trp Ile Ser Asp Thr Glu Phe Ile Tyr Arg Glu
145                 150                 155                 160

Gln Lys Gly Thr Val Arg Leu Trp Asn Val Glu Thr Asn Thr Ser Thr
                165                 170                 175

Val Leu Ile Glu Gly Lys Lys Ile Glu Ser Leu Arg Ala Ile Arg Tyr
            180                 185                 190

Glu Ile Ser Pro Asp Arg Glu Tyr Ala Leu Phe Ser Tyr Asn Val Glu
        195                 200                 205

Pro Ile Tyr Gln His Ser Tyr Thr Gly Tyr Tyr Val Leu Ser Lys Ile
    210                 215                 220
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Pro His Gly Asp Pro Gln Ser Leu Asp Pro Pro Glu Val Ser Asn Ala
225                 230                 235                 240

Lys Leu Gln Tyr Ala Gly Trp Gly Pro Lys Gly Gln Gln Leu Ile Phe
                245                 250                 255

Ile Phe Glu Asn Asn Ile Tyr Tyr Cys Ala His Val Gly Lys Gln Ala
            260                 265                 270

Ile Arg Val Val Ser Thr Gly Lys Glu Gly Val Ile Tyr Asn Gly Leu
        275                 280                 285

Ser Asp Trp Leu Tyr Glu Glu Glu Ile Leu Lys Thr His Ile Ala His
    290                 295                 300

Trp Trp Ser Pro Asp Gly Thr Arg Leu Ala Tyr Ala Ala Ile Asn Asp
305                 310                 315                 320

Ser Arg Val Pro Ile Met Glu Leu Pro Thr Tyr Thr Gly Ser Ile Tyr
                325                 330                 335

Pro Thr Val Lys Pro Tyr His Tyr Pro Lys Ala Gly Ser Glu Asn Pro
            340                 345                 350

Ser Ile Ser Leu His Val Ile Gly Leu Asn Gly Pro Thr His Asp Leu
        355                 360                 365

Glu Met Met Pro Pro Asp Asp Pro Arg Met Arg Glu Tyr Tyr Ile Thr
    370                 375                 380

Met Val Lys Trp Ala Thr Ser Thr Lys Val Ala Val Thr Trp Leu Asn
385                 390                 395                 400

Arg Ala Gln Asn Val Ser Ile Leu Thr Leu Cys Asp Ala Thr Thr Gly
                405                 410                 415

Val Cys Thr Lys Lys His Glu Asp Glu Ser Glu Ala Trp Leu His Arg
            420                 425                 430

Gln Asn Glu Glu Pro Val Phe Ser Lys Asp Gly Arg Lys Phe Phe Phe
        435                 440                 445

Ile Arg Ala Ile Pro Gln Gly Gly Arg Gly Lys Phe Tyr His Ile Thr
    450                 455                 460

Val Ser Ser Ser Gln Pro Asn Ser Ser Asn Asp Asn Ile Gln Ser Ile
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465                 470                 475                 480

Thr Ser Gly Asp Trp Asp Val Thr Lys Ile Leu Ala Tyr Asp Glu Lys
                485                 490                 495

Gly Asn Lys Ile Tyr Phe Leu Ser Thr Glu Asp Leu Pro Arg Arg Arg
            500                 505                 510

Gln Leu Tyr Ser Ala Asn Thr Glu Gly Asn Phe Asn Arg Gln Cys Leu
        515                 520                 525

Ser Cys Asp Leu Val Glu Asn Cys Thr Tyr Phe Ser Ala Ser Phe Ser
    530                 535                 540

His Ser Met Asp Phe Phe Leu Leu Lys Cys Glu Gly Pro Gly Val Pro
545                 550                 555                 560

Met Val Thr Val His Asn Thr Thr Asp Lys Lys Lys Met Phe Asp Leu
                565                 570                 575

Glu Thr Asn Glu His Val Lys Lys Ala Ile Asn Asp Arg Gln Met Pro
            580                 585                 590

Lys Val Glu Tyr Arg Asp Ile Glu Ile Asp Asp Tyr Asn Leu Pro Met
        595                 600                 605

Gln Ile Leu Lys Pro Ala Thr Phe Thr Asp Thr Thr His Tyr Pro Leu
    610                 615                 620

Leu Leu Val Val Asp Gly Thr Pro Gly Ser Gln Ser Val Ala Glu Lys
625                 630                 635                 640

Phe Glu Val Ser Trp Glu Thr Val Met Val Ser Ser His Gly Ala Val
                645                 650                 655

Val Val Lys Cys Asp Gly Arg Gly Ser Gly Phe Gln Gly Thr Lys Leu
            660                 665                 670

Leu His Glu Val Arg Arg Arg Leu Gly Leu Leu Glu Glu Lys Asp Gln
        675                 680                 685

Met Glu Ala Val Arg Thr Met Leu Lys Glu Gln Tyr Ile Asp Arg Thr
    690                 695                 700

Arg Val Ala Val Phe Gly Lys Asp Tyr Gly Gly Tyr Leu Ser Thr Tyr
705                 710                 715                 720
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Ile Leu Pro Ala Lys Gly Glu Asn Gln Gly Gln Thr Phe Thr Cys Gly
725 730 735

Ser Ala Leu Ser Pro Ile Thr Asp Phe Lys Leu Tyr Ala Ser Ala Phe
740 745 750

Ser Glu Arg Tyr Leu Gly Leu His Gly Leu Asp Asn Arg Ala Tyr Glu
755 760 765

Met Thr Lys Val Ala His Arg Val Ser Ala Leu Glu Glu Gln Gln Phe
770 775 780

Leu Ile Ile His Pro Thr Ala Asp Glu Lys Ile His Phe Gln His Thr
785 790 795 800

Ala Glu Leu Ile Thr Gln Leu Ile Arg Gly Lys Ala Asn Tyr Ser Leu
805 810 815

Gln Ile Tyr Pro Asp Glu Ser His Tyr Phe Thr Ser Ser Ser Leu Lys
820 825 830

Gln His Leu Tyr Arg Ser Ile Ile Asn Phe Phe Val Glu Cys Phe Arg
835 840 845

Ile Gln Asp Lys Leu Pro Thr Val Thr Ala Lys Glu Asp Glu Glu Glu
850 855 860

Asp
865

<210> 10
<211> 41
<212> PRT
<213> Homo sapiens

<400> 10

Ile Leu Ala His His Gly Glu Ala Asp Pro Phe Val Pro Ala Glu Ala
1 5 10 15

Val Asp Gln Leu Glu Glu Ala Leu Arg Ala Ala Asn Val Asp Leu Glu
20 25 30

Ile His Val Tyr Pro Gly Ala Gly His
35 40